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Discovery of novel 2-substituted-4-(2-fluorophenoxy) pyridine derivatives possessing pyrazolone and triazole moieties as dual c-Met/VEGFR-2 receptor tyrosine kinase inhibitors

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ABSTRACT

In our efforts to develop novel dual c-Met/VEGFR-2 inhibitors as potential anticancer agents, a series of 2-substituted-4-(2-fluorophenoxy) pyridine derivatives bearing pyrazolone scaffold were designed and synthesized. The cell proliferation assay *in vitro* demonstrated that most target compounds had inhibition potency on both c-Met and VEGFR-2, especially compound **9h**, **12b** and **12d**. Based on the further enzyme assay *in vitro*, compound **12d** was considered as the most promising one, the IC_{50} values of which were 0.11 μM and 0.19 μM for c-Met and VEGFR-2, respectively. Further molecular docking studies suggested a common mode of interaction at the ATP-binding site of c-Met and VEGFR-2, indicating that **12d** was a potential compound for cancer therapy deserving further study.

1. Introduction

Cancer is a complex, universal, and fatal disease. There were 8.2 million deaths, 14.1 million new cancer cases and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [1]. It was also predicted that global cancer cases could increase to 22 million per year by 2030 [2]. Therefore, the identification of novel biological targets and the discovery of more specific chemotherapeutics are significant objectives of current research, especially for the most invasive tumors.

Receptor tyrosine kinases (RTKs) play critical roles in numerous cellular processes and signal transduction pathways. When mutated or overexpressed, RTKs become potent oncoproteins that can cause deregulated cell growth, angiogenesis and metastasis [3], making them key targets for small molecule inhibitors in the treatment of cancer. Several RTK inhibitors have been found to have effective anti-tumor activity and some of them are in clinical trials or have been approved [4].c-Mesenchymal epithelial transition factor (c-Met), a tyrosine kinase receptor for hepatocyte growth factor (HGF), is widely over-expressed in human tumors. Binding of HGF to c-Met induces phosphorylation of tyrosine residues on c-Met and activates its downstream signaling pathway which implicates in cell proliferation, invasion, metastasis, and angiogenesis of various tumors [5,6]. Importantly, abnormal c-Met activation observed frequently in many human solid tumors and hematological malignancies is related to poor clinical outcomes [7]. Furthermore, overactivation of c-Met causes therapeutic resistance [8]. Consequently, inhibition of c-Met activity is a potentially impactful approach to the treatment of cancers where c-Met is activated [9]. On the basis of the analysis of binding modes, the small molecular inhibitors of c-Met can be classified into different types. One important type of c-Met inhibitors is multikinase inhibitors and usually strongly inhibits VEGFR and other homological kinases as well [10].

Vascular endothelial growth factor receptor 2 (VEGFR-2, also known as KDR) is a tyrosine kinase receptor expressed in endothelial cells. Binding of VEGF to VEGFR induces a conformational change in VEGFR, followed by receptor dimerization and phosphorylation of tyrosine residues. VEGF signaling through VEGFR-2 has been shown to play a major role in the regulation of tumor angiogenesis [11,12]. Expression of VEGF is enhanced in several types of human tumors, and its expression levels are associated with poor prognosis and clinical stage in patients with solid tumors

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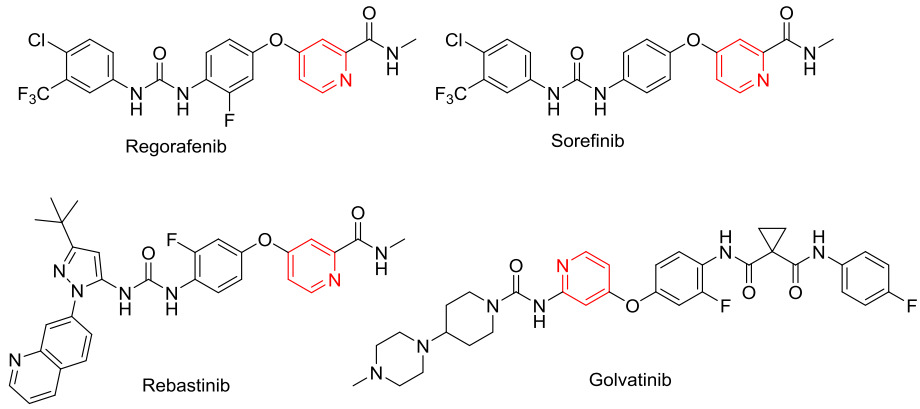

Fig. 1. Kinase inhibitors using pyridine skeleton.

[13–20]. Therefore, VEGF/VEGFR-2 signaling is an attractive therapeutic target in the treatment of cancer.c-Met has been shown to synergistically collaborate with VEGFR-2, resulting in promoting angiogenesis of development and progression of various human cancers [21–23]. Thus, molecules that simultaneously inhibit c-Met and VEGFR-2 may be superior to either c-Met-selective or VEGFR-2-selective inhibitor as they can interrupt multiple signaling pathways involved in tumor proliferation, metastasis and angiogenesis [24]. Several kinase inhibitors, including cabozantinib [25] and foretinib [26], were previously reported to suppress c-Met and VEGFR-2 kinases simultaneously. We therefore started the synthetic studies of dual c-Met and VEGFR-2 TKIs with potent antitumor efficacy exploiting pyridine nucleus widely used in drug development, especially in the receptor tyrosine kinase inhibitor.

As shown in Fig. 1, the pyridine skeleton had been widely used in VEGFR inhibitors, c-Met inhibitors, multiple target kinase inhibitors and Bcr-Abl kinase inhibitors. Thus we introduced pyridine skeleton to the initial compound (**I** in Fig. 2). Our docking study of the pyridine nucleus revealed that it could penetrate into the ATP binding pocket of the c-Met and VEGFR-2 receptors (PDB code: 3LQ8, 3U6J), and the nitrogen atom of pyridine ring could interact with the amino acid residues (Met1160 of the c-Met protein and Cys919 of the VEGFR-2) in the hinge region. At the same time, a pyrazolone ring was introduced to the side chain moiety (**I** in Fig. 2), which conformed to '5 atoms regulation' [27] and might form hydrogen bonds with amino acid residues of c-Met and VEGFR-2. In order to obtain novel dual c-Met/ VEGFR-2 inhibitors, we then cyclized the amide bond to give a triazole fragment (**II** in Fig. 2). The docking studies also showed that the triazole could penetrate into the hydrophilic channel of c-Met and VEGFR-2, and the N atom of the triazole could generate a new H bond with Tyr1159 of VEGFR-2. A series of new 2-substituted-4-(2-fluorophenoxy) pyridine derivatives possessing pyrazolone moiety were synthesized and their inhibitory activities were evaluated. Furthermore, the preliminary structure–activity relationships and possible enzyme binding modes were also illustrated.

2. Results and discussion

2.1. Chemistry

The key intermediate **2** was prepared by Vilsmeier-Haack reaction [28] of antipyrine and DMF/$POCl_3$ under room temperature. To prepare compound **7**, the requisite pyrazolone carboxylic acid **3** was synthesized through a Pinnick oxidation [29] of aldehyde **2** (Scheme 1).

The synthesis of 2-substituted-4-(2-fluorophenoxy) pyridine derivatives **7**, **9a-9o**, **12a-12d** is shown in Scheme 2. A nucleophilic substitution of methyl 4-chloropicolinate with 2-fluoro-4-nitrophenol in refluxing chlorobenzene provided **5** in a moderate yield [30]. The amino intermediate **6** was afforded by the reduction in intermediate **5** in the presence of Pd/C, and was then reacted with pyrazolone carboxylic acid **3** to generate compound **7**. Hydrolysis of **7** with lithium hydroxide monohydrate in THF/MeOH/H_2O led to the desired acid **8**, which then reacted with various amines to obtain compounds **9a-9o.** A convenient hydrazinolysis of **7** in MeCN resulted in **10**, which then reacted with N, N-dimethylformamide dimethyl acetal (DMF-DMA) to generate **11** in a high yield. The proposed compounds **12a-12d** were generated by cyclization of **11** with the appropriate amines in the presence of acetic acid [31].

2.2. Cell proliferation inhibition of target compounds

The effects of the compounds on cell proliferation were evaluated using BaF3-TPR-Met cells which were the c-Met-dependent cell line, and in VEGF-stimulated human umbilical vein endothelial cells (HUVEC).

All compounds depicted in Tables 1and 2 were firstly screened for inhibition against BaF3-TPR-Met. Also included were the activities of reference compound Cabozantinib. Among the tested compounds, **9h**, **9j**, **9l**, **9o**, **12b**, **12c** and **12d** showed the most potent inhibitory activities, which were then selected to further test their

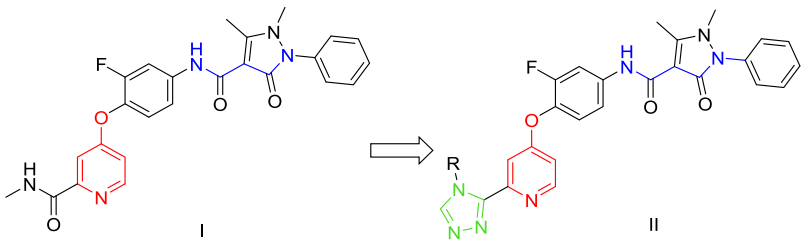

Fig. 2. Design of novel inhibitor for both c-Met and VEGFR-2 kinases.